How cationic lipids offer new possibilities in the delivery of RNA therapeutics

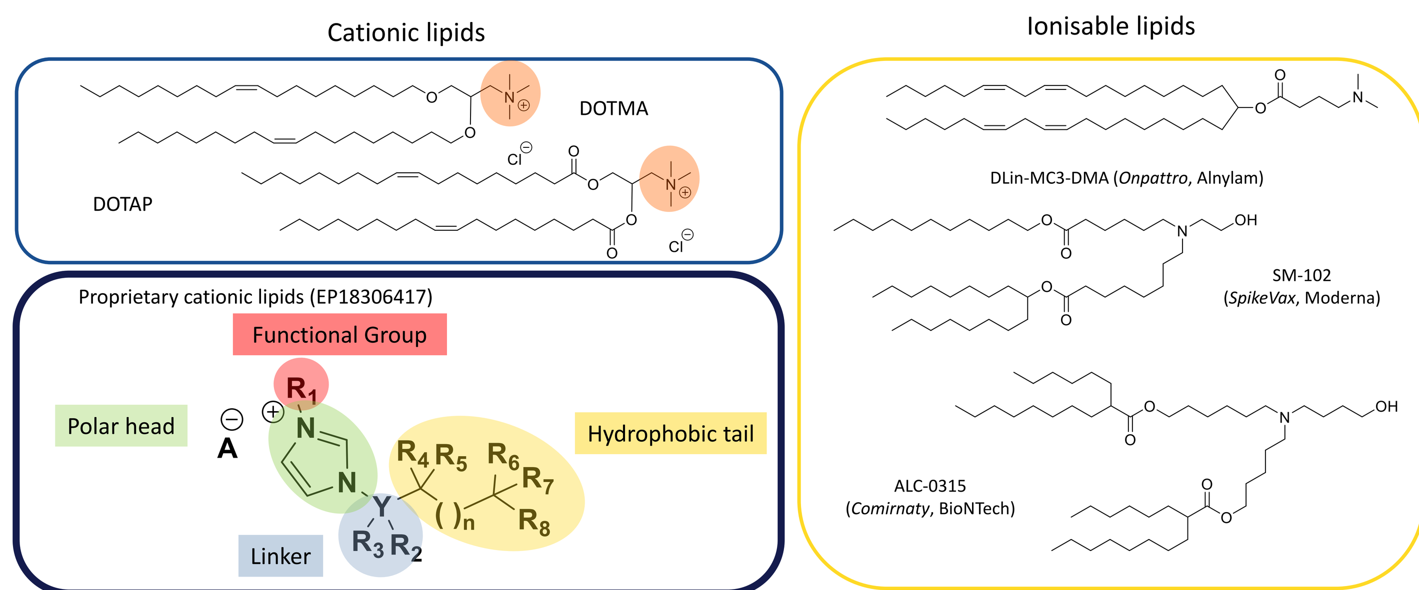
Polyplus®

Claire Guéguen, Malik Hellal, Thibaut Benchimol, Safiyat Amine, Mélodie Seiler, Margaux Briand, Morgane Ziesel, Kassandra Renaud, Patrick Erbacher
Polyplus-transfection®, Vectura, 75 rue Marguerite Perey, 67400 Illkirch, France

Abstract

Ionizable lipid nanoparticles (LNPs) have been widely used for *in vivo* delivery of RNA therapeutics with the particular that they predominantly end up targeting the liver (Patisiran, BNTech162b, mRNA-1273). The current challenge is the use of commercially available lipids to have a better control over the biodistribution of the RNA once delivered systemically to target different organs and cell types. Novel lipidic formulations using different cationic lipids were characterized by DLS to assess size and zeta potential and mRNA encapsulation efficiency was assessed by the RiboGreen assay. Novel lipidic formulations were tested for mRNA delivery both *in vitro* and *in vivo* though intra-veinous and intramuscular injections to evaluate their stability, efficacy and biodistribution.

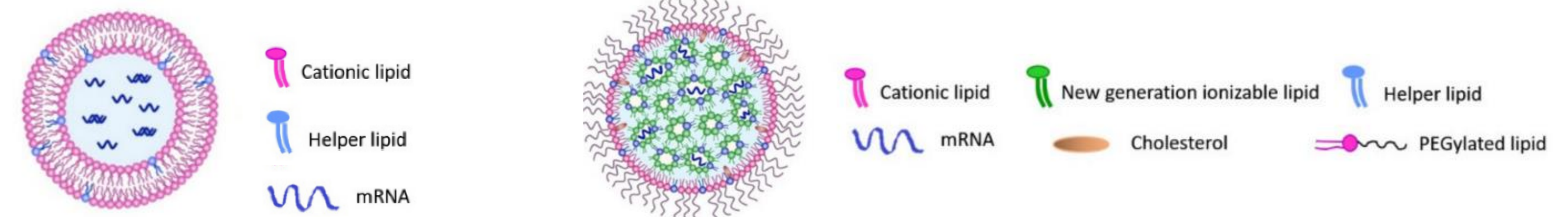
Cationic vs ionisable



Classical cationic lipids used for LNPs (DOTMA and DOTAP) have a tri-methyl ammonium part which can induce toxicity. The alternative is to use ionisable lipids (Dlin-MC3-DMA, SM-102 or ALC-0315) but with a restrictive biodistribution and stability. Our library of cationic lipid is different from the classical cationic lipids mainly due to the polar head which is an imidazolium and also to the presence of a R1 functional group which hides the positive charge and then could also reduce the toxicity.

Liposomes vs LNPs

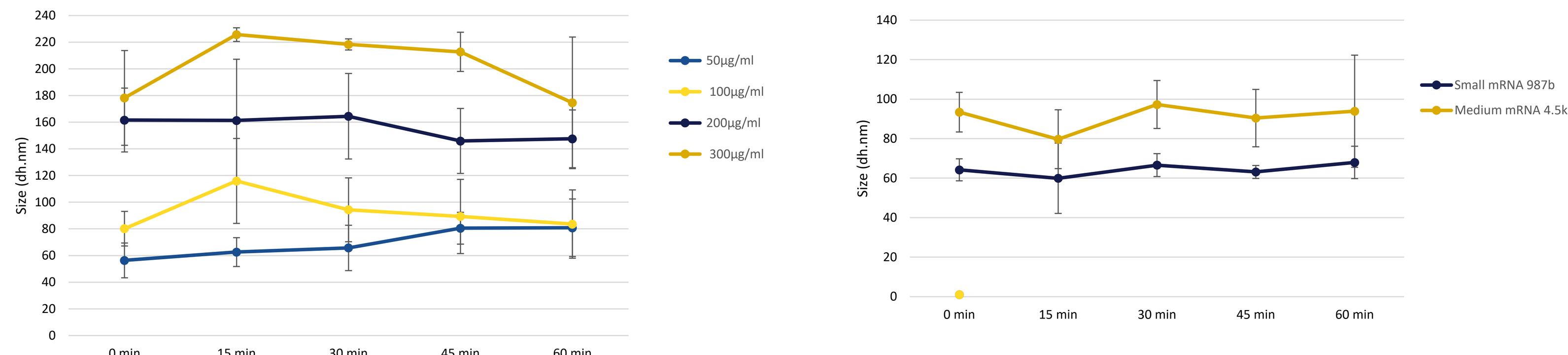
- mRNA liposomes
  - Off the shelf product
  - Pipette mixing liposomes:
    - jetMESSENGER®
    - in vivo-jetRNA®+
    - + mRNA
- mRNA-LNP (Lipid nanoparticle)
  - Made with microfluidic technology (NanoAssemblr)
  - by mixing lipids including our proprietary cationic lipid (Patent n° EP18306417)



Pharmaceutics **2021**, 13, 206. https://doi.org/10.3390/pharmaceutics13020206

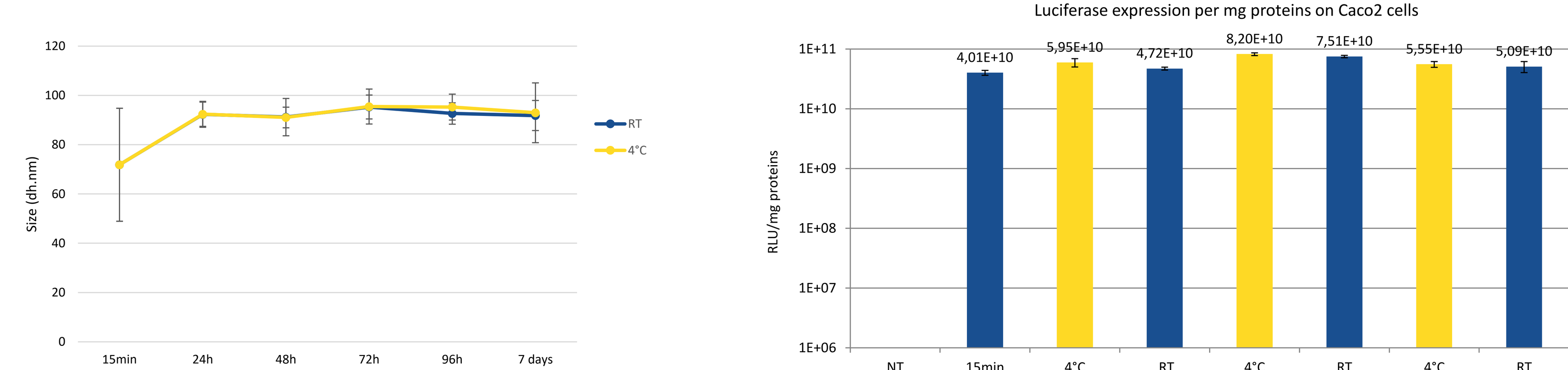
Liposomes are stable at high concentration up to 7 days

- Liposomes with *in vivo*-jetRNA®+ are stable with up to 300 µg of mRNA/mL with different size of mRNA



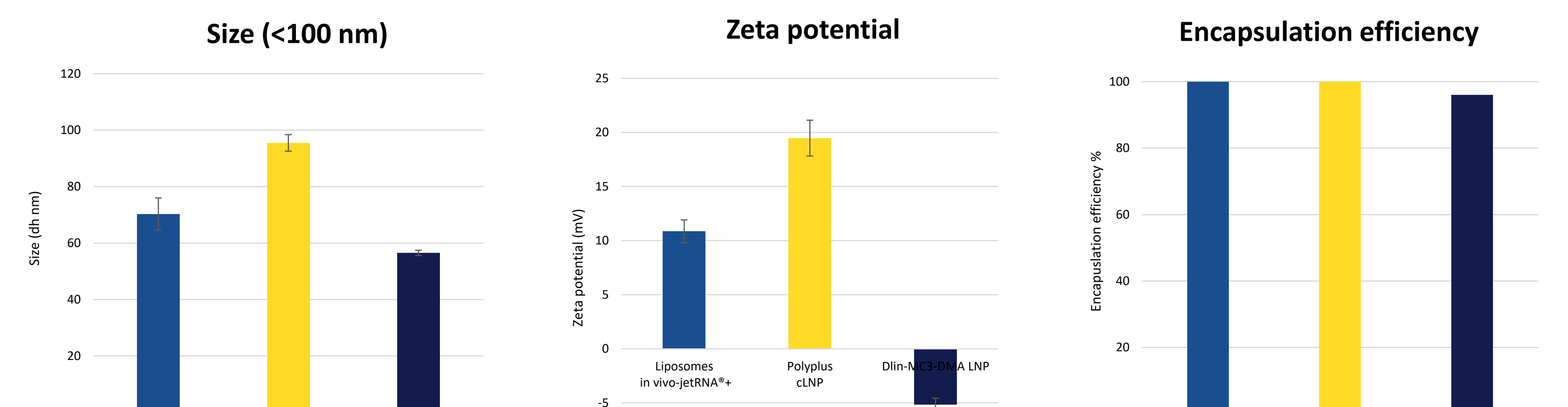
Size of liposomes with *in vivo*-jetRNA®+ at 50, 100, 200 or 300 µg of mRNA/ml with a small mRNA (1929b) or at 50 µg of mRNA/ml with a small size mRNA (978b) or a medium size mRNA (4.5kb) after 15, 30, 45 and 60 min of complexation were measured by dynamic light scattering (DLS).

- Liposomes with *in vivo*-jetRNA®+ are stable up to 7 days



Size of liposomes with *in vivo*-jetRNA®+ at 50 µg/ml after 15 min, 24, 48, 72 or 96 hours or 7 days of complexation were measured with the DLS. Caco-2 were transfected with liposomes formed with a mRNA/*in vivo*-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer. 500 ng of mRNA encoding Luciferase were used for 40,000 Caco2 cells. Luciferase was assessed 24 h after transfection.

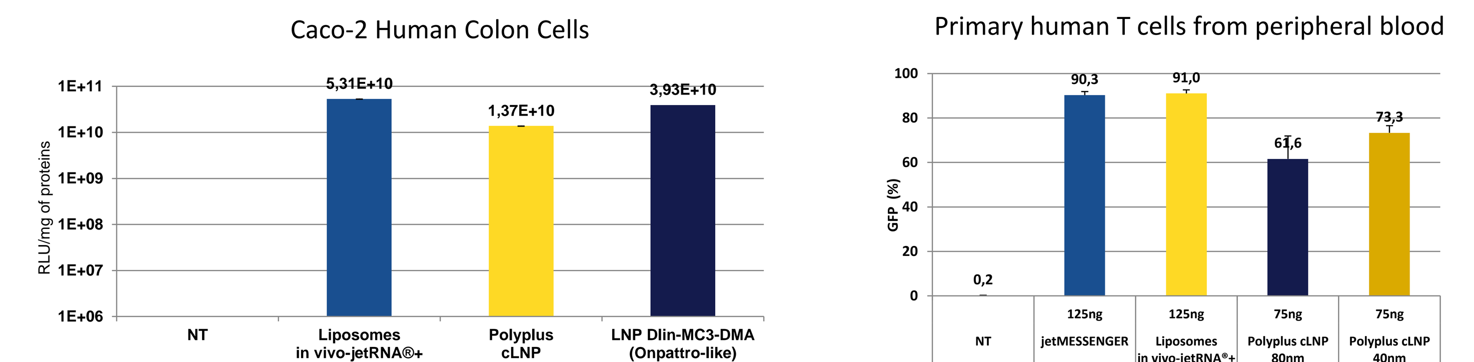
One cationic lipid – two delivery systems with similar behaviours



Size and zeta potential of liposomes with *in vivo*-jetRNA®+ at 50 ng/µl after 1h of complexation or LNPs at 250 ng/µl were measured by DLS. Encapsulation efficiency was assessed by the RiboGreen assay.

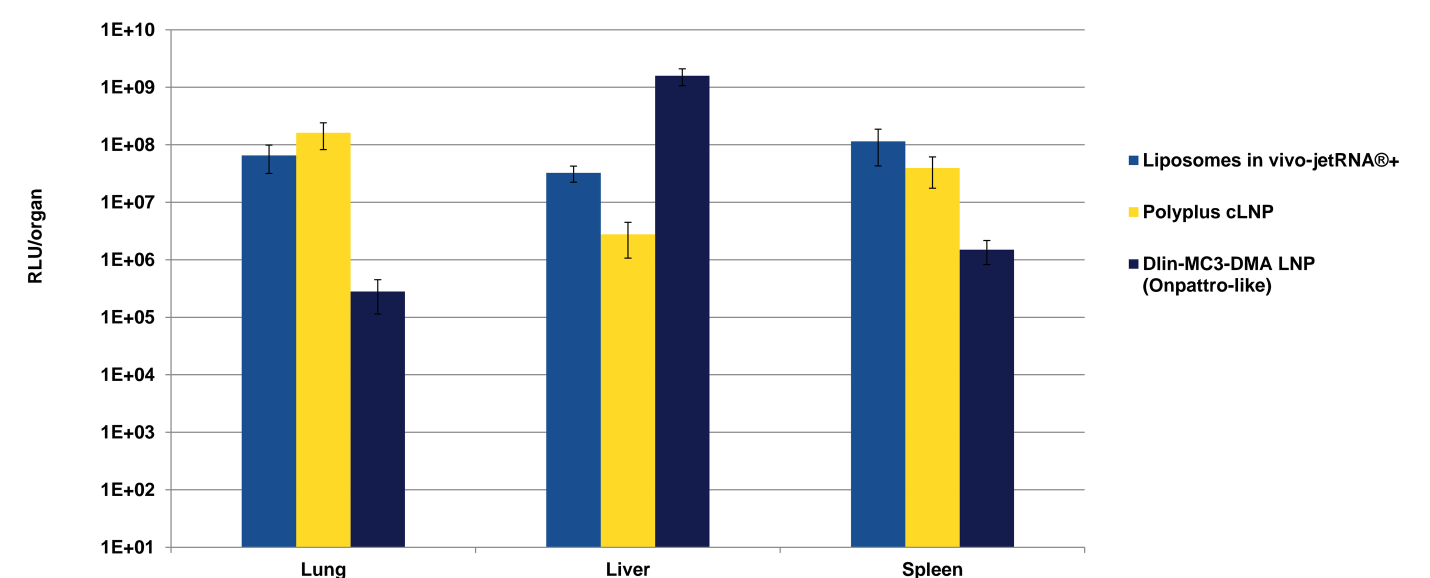
Cationic LNPs: different biodistribution than neutral LNPs

- *In vitro* transfection efficacy



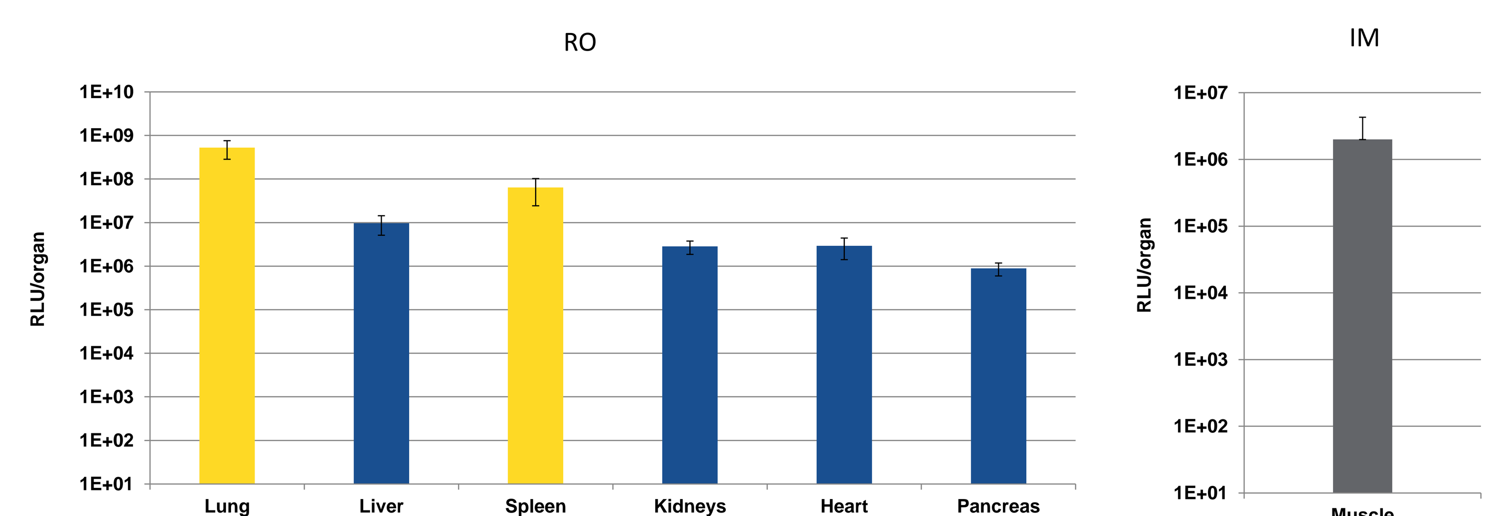
Caco-2 and primary human T cells were transfected with liposomes formed with a mRNA/*in vivo*-jetRNA®+ or jetMESSENGER® ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer or LNPs using either 500 ng or 75 and 125 ng of mRNA encoding Luciferase or GFP for respectively 40,000 Caco2 cells or 187,500 T cells. Luciferase or GFP expression was assessed 24 h or 48 h after transfection for respectively Caco-2 cells or T cells.

- Different expression pattern profile with Polyplus positively charged LNP compared to Dlin-MC3-DMA LNP



mRNA encoding Luciferase was injected into mice using *in vivo*-jetRNA®+ or Polyplus cLNP or Dlin-MC3-DMA LNP through intravenous injection. Liposomes were formed using 10 µg of mRNA with an mRNA/*in vivo*-jetRNA® ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer. Luciferase expression was assessed 24 h post-injection.

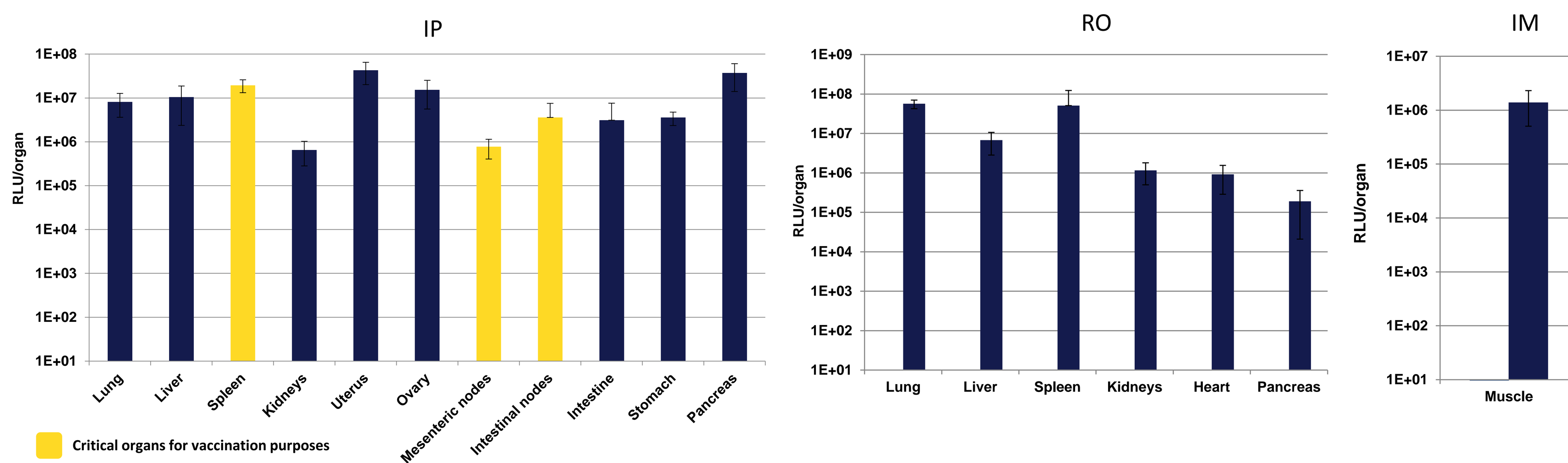
- Polyplus cLNP mainly targets lung and spleen



mRNA encoding Luciferase was injected into mice using Polyplus cLNP through different administration routes. 10 µg mRNA were injected for intravenous injection (retro-orbital injection – RO) or 5 µg mRNA for intramuscular (IM) injection. Luciferase expression was assessed 24 h post-injection.

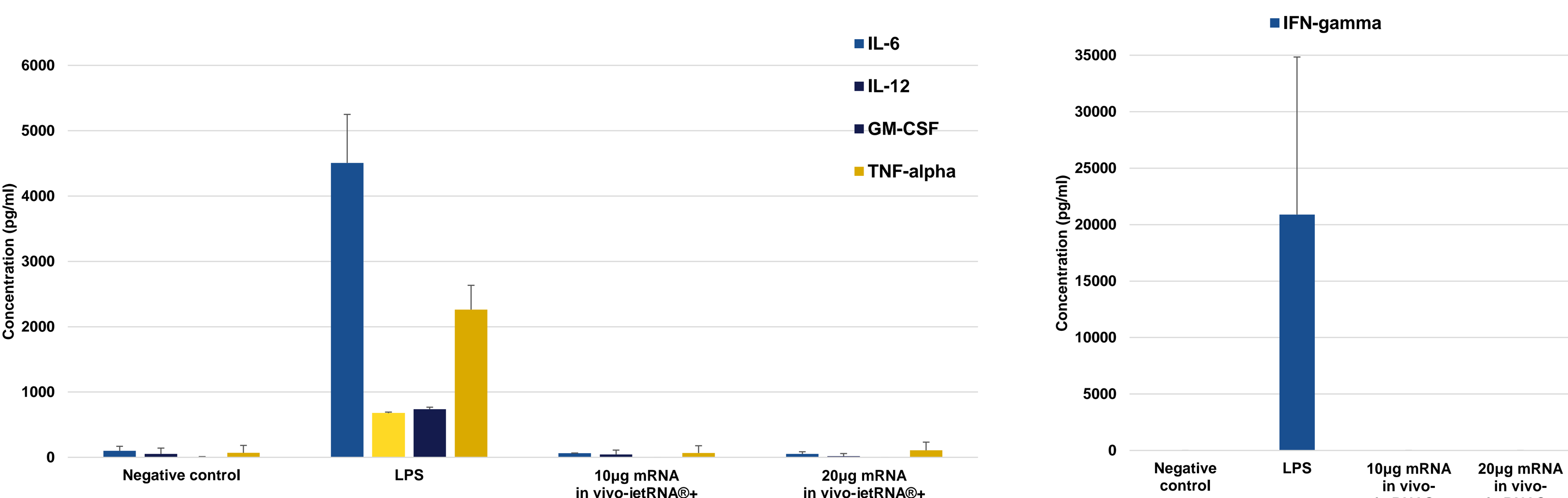
Liposomes: efficient and safe mRNA delivery for local and systemic administration

- *In vivo* efficacy and biodistribution



mRNA encoding Luciferase was injected into mice using *in vivo*-jetRNA®+ through different administration routes. Complexes were formed with a mRNA/*in vivo*-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) in mRNA Buffer using either 20 µg mRNA for intraperitoneal (IP) injection, 10 µg mRNA for intravenous (retro-orbital injection – RO) or 5 µg mRNA for intramuscular (IM) injection. Luciferase expression was assessed 24 h post-injection.

- No pro-inflammatory cytokine expression



mRNA complexes were formed in 200 µL of mRNA Buffer using 10 or 20 µg of mRNA encoding Luciferase at a mRNA/*in vivo*-jetRNA®+ ratio of 1:2 (μg_{mRNA}:$\mu L_{reagent}$) and injected through intravenous injection (retro-orbital injection). 2 to 24 hours after injection, blood was collected and the level of IL-6, IL-12, GM-CSF, IFN-gamma and TNF-alpha was measured by ELISA (IL-6) or MACSPlex kits. As a positive control, LPS (200 µg) was administered into mice.

Conclusion

Our novel proprietary cationic lipid solves a current limit of LNPs to target different organs and cell types. Our novel lipidic formulations ensure the same efficacy as LNPs with ionizable lipids, while ensuring better biodistribution to target organs other than liver.